

Development of the Larval Serotonergic Nervous System in the Sea Star *Patriella regularis* as Revealed by Confocal Imaging

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Abstract. Development of the nervous system in the larvae of the sea star *Patriella regularis* was reconstructed in three dimensions. The optical sectioning and image processing capabilities of the confocal microscope made it possible to identify the precise location and timing of development of serotonergic cells in relation to subsequent development of larval features. Similarities between this system and the serotonergic systems in larvae of other echinoderms were explored. Neuronal-like immunoreactive cells and processes first appeared in late gastrulae as a collection of cells scattered across the animal pole. These cells subsequently gave rise to basal axons positioned along the basal lamina. Immunopositive cells located in the stomodaeal region marked the beginnings of formation of the adoral ciliated band. Cells were also present in the mid-dorsal epithelium. Advanced bipinnaria had pyramidal immunoreactive cells within the adoral band and ovoid immunoreactive cells within the preoral and postoral ciliated bands. Processes originating from neurons in the transverse region of the preoral ciliated band extended into the buccal cavity, suggesting that these cells have a sensory role in feeding. An anterior ganglion formed in the late bipinnaria, innervating the preoral and postoral ciliated bands. This connection has not previously been described. It thus appears that the ciliated bands in the bipinnaria larvae of *P. regularis* communicate *via* serotonergic nerve tracts.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is a ubiquitous monoamine and functions as a neurotransmitter in the adult

nervous systems of a large range of animal groups (Collier, 1958). In echinoderms, serotonin was first isolated in the gonads of adult asteroids (Welsh and Moorehead, 1960), and its cellular location has been documented in studies of the larval nervous system of echinoids, asteroids, and holothuroids (Bisgrove and Burke, 1986; Burke *et al.*, 1986; Bisgrove and Burke, 1987; Nakajima, 1988; Bisgrove and Raff, 1989; Nakajima *et al.*, 1993; Moss *et al.*, 1994; Chee and Byrne, 1997). These studies indicate that serotonin functions in a neuronal capacity in these larvae. The feeding larvae of echinoderms typically have a well-developed serotonergic nervous system that innervates the ciliated bands and is suggested to play a sensory role in feeding and metamorphosis. The similarities between the serotonergic systems in the larvae of several echinoderm classes have been taken to suggest that these systems are homologous (Burke *et al.*, 1986). In general, the increasing complexity of the serotonergic nervous system in the feeding larvae of echinoderms parallels the development of the ciliated bands (Burke, 1983).

During sea star development the number of serotonergic immunoreactive cells increases from a few cells at the gastrula stage to a complex nervous system in competent larvae prior to metamorphosis (Nakajima, 1988). In this study we investigate the larval nervous system of the sea star *Patriella regularis*. This species has planktotrophic development through bipinnaria and brachiolaria feeding stages (Byrne and Barker, 1991). Following from previous observations (Chee and Byrne, 1997), we document the expression of serotonin in *P. regularis* from the first appearance of neurons in gastrulae through the formation of a complex three-dimensional nervous system. Assisted by confocal microscopy, we were able to reconstruct development of the serotonergic-like nervous system in three dimensions with respect to morphogenetic change. Although

Received 2 November 1998; accepted 28 June 1999.

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Abbreviations: ADNP, adoral nerve plexus; FSW, filtered seawater; PBS, phosphate buffered saline; AG, anterior ganglion; 5-HT, serotonin.

previous studies have successfully used epifluorescence microscopy to follow the formation of the larval serotonergic nervous system in echinoderms (Burke, 1983; Nakajima, 1988; Moss *et al.*, 1994), insights into cell structure and the three-dimensional nature of the system were limited with this technique.

For echinoderms, development through feeding larvae is considered to be the ancestral life-history pattern (Strathmann, 1978; Hart *et al.*, 1997). In *Patiriella*, developmental evolution has resulted in the loss of a feeding larva and adaption of various forms of planktotrophic, benthic, in-tragonadal, and lecithotrophic larvae (Byrne and Cerra, 1996; Hart *et al.*, 1997). A major aim of this study was to obtain a more complete picture of 5-HT-like expression in the ancestral-type larvae of *P. regularis* and demonstrate that during the development of *P. regularis* small changes in gross morphology coincide with large changes in serotonergic architecture. The significance of the distribution of serotonergic neurons is assessed with respect to the functional morphology of the larvae and the roles these neurons may play in modulating larval behavior. Parallels in the immunocytochemical expression of 5-HT with other neurotransmitters in the feeding larvae of other echinoderms are discussed. The nomenclature used to describe the ciliated bands follows that established by previous authors (Strathmann, 1975; Moss *et al.*, 1994).

Materials and Methods

Patiriella regularis were collected from the Derwent Estuary, Tasmania. Mature oocytes were obtained by intracoelomic injection of the starfish with 10^{-5} M 1-methyladenine (Sigma) in 0.2- μ m filtered seawater (FSW). Testes were dissected from mature males, and a dilute solution of sperm was added to the eggs. Fertilization success was visually checked after 15 min. The fertilized eggs were washed three times in FSW to remove any remaining sperm. Embryos and larvae were cultured at 22°C in FSW, and the larvae were fed *Dunaliella tertiolecta*, *Rhodomonas* sp., or both.

Gastrulae, early and late bipinnariae, and early brachiolariae were immunolabeled for microscopic examination. Gastrulae were obtained from four cultures derived from different fertilizations. The bipinnaria were derived from six different cultures. All of the stages were transferred to glass scintillation vials, fixed in 4% paraformaldehyde in FSW at 22°C for 1–2 h, rinsed briefly in FSW, and then placed into phosphate buffered saline (PBS) at pH 8.2–8.3. Prior to antibody incubation, specimens were treated for 30 min in PBS containing normal goat serum and 0.3% Triton \times 100 to reduce nonspecific staining and to aid antibody penetration. After each incubation step the specimens were washed with gentle agitation in three 10-min changes of 0.1 M PBS pH 8.2. Specimens were then incubated first in the primary

antibody, rabbit anti-serotonin (Incstar/DiaSorin) diluted 1 in 100, for 16 to 22 h at 4°C, and then in the secondary antibody, biotinylated goat anti rabbit IgG (H+L) (Vector Laboratories) 1 in 50, for 2 h. The final incubation was in a 1-in-100 dilution of fluorescein (FITC)-labeled streptavidin (Vector Laboratories) for 20 min in the dark at 23°C. Controls consisted of omitting the primary antibody, omitting the secondary antibody, using normal rabbit serum as a substitute for the primary antibody, and checking for autofluorescence using only paraformaldehyde-fixed gastrulae and larvae. Immunolabeled gastrulae ($n > 100$) and larvae were mounted on wetted slides in a drop of Fluoroguard antifade reagent (Bio-Rad), and the coverslips were sealed with nail polish. Slides were viewed immediately or stored at 4°C in the dark. Slides refrigerated for several months showed no sign of fading when examined. All times quoted are postfertilization.

The specimens were examined with a confocal laser scanner coupled to an epifluorescence microscope (a Bio Rad MRC600 scanner and a Zeiss Axiophot microscope or an MRC1024 and an Olympus BX 60). The 488-nm line of the krypton/argon laser was used with a 520DF32-nm filter block. Various numbers of optical sections were collected at different depth intervals. The depth of collection was determined by the thickness of the specimen and the degree of immunolabeling. The number of immunoreactive cell bodies was determined by optically sectioning the various larvae. All images are displayed in ventral or lateral view, anterior at the top of the page. Image projections (extended focal length) were created using Confocal Assistant (Software version 4.02), and three-dimensional (3D) stereo anaglyphs were produced using Laser Sharp (Bio-Rad Laboratories) and Confocal Assistant. Computer animations were produced using a Silicon Graphics XS24 4000 with Voxel View Ultra software.

Patiriella regularis larvae were prepared for scanning electron microscopy according to Byrne and Barker (1991) and examined with a Philips XL30 at 10 Kv.

Results

Gastrula

The first cells exhibiting specific 5-HT-like immunoreactivity occurred in mid gastrulae (Fig. 1A), about 24 h postfertilization. As the gastrulae began to elongate, these cells formed a partial dome-like array across the animal region and included monopolar, bipolar, and tripolar cells (Fig. 1B). Varicosities were occasionally observed on these processes (Fig. 1C). Both the soma and the neurites of these cells were immunopositive (Fig. 1C). In cross section the cells spanned the epithelium (Fig. 1D). With 3D computer reconstructions or extended focus projections, these cells were shown to be pyramidal. Control gastrulae ($n = 15$), were nonfluorescent.

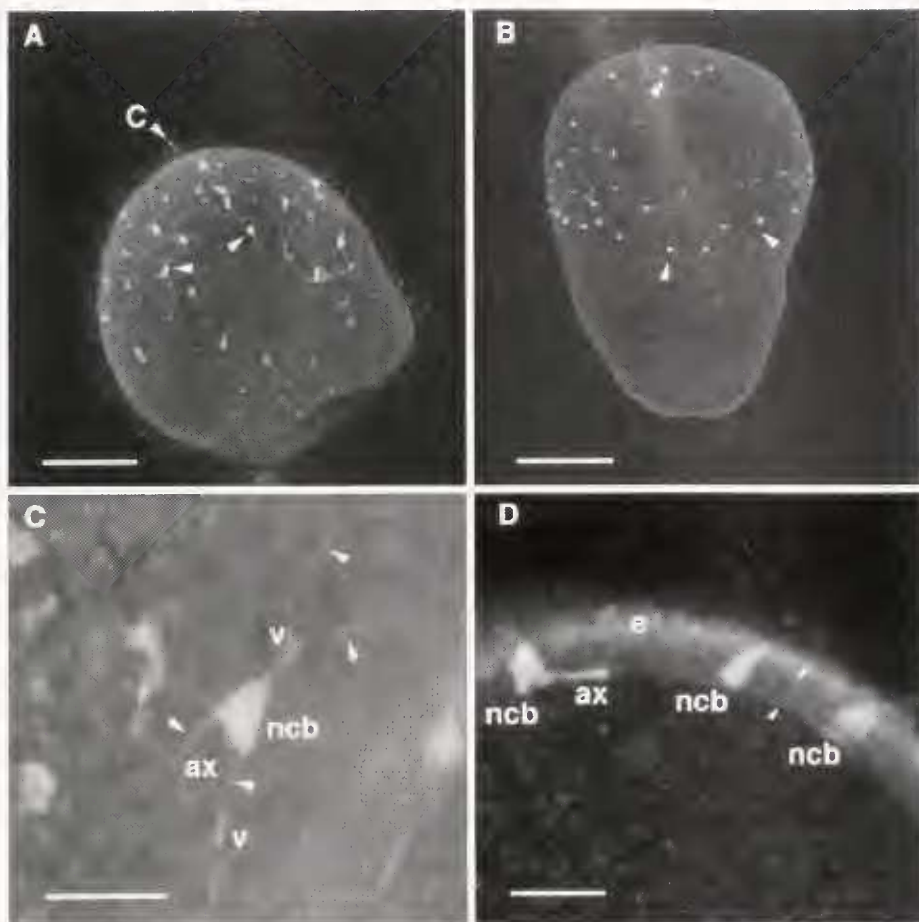


Figure 1. Confocal images showing bodies and processes of cells with 5-HT-like immunoreactivity in early and advanced gastrula. (A) A projection from 16 optical sections taken at 4.5- μm intervals of a mid gastrula shows cells with 5-HT-like immunoreactivity (arrowheads) scattered in the epithelium. C, cilia. Bar, 63 μm . (B) Advanced gastrula, projection created from 14 images at 4.5- μm depth intervals showing the concentration of immunoreactive cells (arrowheads) in the animal half. Bar, 95 μm . (C) 5-HT-like immunoreactivity in a tripolar nerve cell in a 34-h gastrula. ax, axonal-like processes; v, varicosities; ncb, nerve cell body. Bar, 16 μm . (D) Advanced gastrula/early bipinnaria epithelium (e) showing nerve cell bodies (ncb) and axonal-like processes (ax) traveling along the basal lamina in a single confocal section. Bar, 20 μm .

Bipinnaria

Prior to the opening of the mouth, early bipinnariae had a distinct stomodaeal invagination while the blastopore was still located at the vegetal pole (Fig. 2A). The larvae could now be orientated according to their dorsoventral axis (Fig. 2A). A 144- μm -thick projection reconstructed from 32 optical sections showed that cells with 5-HT-like immunoreactivity were abundant on both sides of the larva (Fig. 2A). On the ventral surface, the cells around the stomodaeum, about 10 in number, were monopolar and marked the position at which the adoral ciliated band will form (Fig. 2A). A collection of bipolar ovoid immunoreactive cells on the dorsal surface was positioned roughly opposite the stomodaeal invagination (Fig. 2A).

At about 48 h postfertilization, the mouth opened. The

larvae were further elongated and the anus opened ventrally. With completion of the gut, the larvae were able to feed. As seen above, 5-HT-like immunoreactivity was conspicuous in the cells surrounding the mouth, which marked the position of the developing adoral ciliated band (Fig. 2B). A few immunoreactive cell bodies were also observed on the upper right region of the buccal cavity (Fig. 2B). Immunoreactive cells and processes on the mid-dorsal surface formed an incomplete ring that wrapped partially around the larva but did not extend to the ventral surface (Fig. 2C). Axonal-like processes from these cell bodies extended towards the posterior end of the larva (Fig. 2C). Although the fate of the immunoreactive cells in this ring could not be followed, their mid-body position indicates that they were subsequently

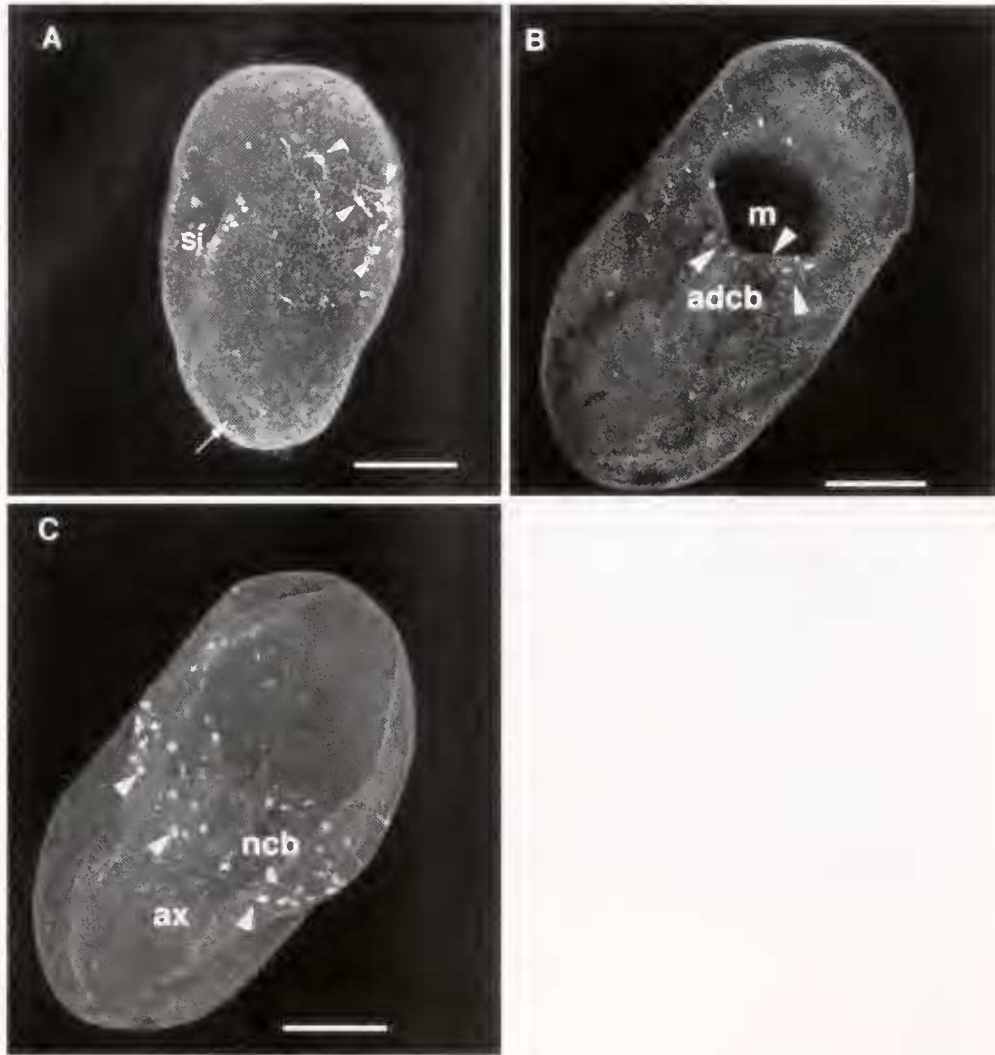


Figure 2. Confocal optical projections of early bipinnariae. (A) Early bipinnaria reconstructed from 32 optical sections. Cells with 5-HT-like immunoreactivity can be clearly seen around the stomodaal invagination (si) and the dorsal surface (arrowheads) of the larva. (B) Projection of 5 confocal sections from the ventral side of a 48-h bipinnaria. 5-HT-like immunoreactivity is present in cell bodies (arrowheads) in the adoral ciliated band (adcb), and a few immunoreactive cells are also present on the upper left-hand side of the mouth (m). (C) Projection of 5 optical sections from the dorsal side of the larva in panel B. Immunoreactive cell bodies (ncb) and axonal-like processes (ax) form a band partially wrapping around the larva. Bars, 95 μ m.

incorporated into the serotonergic tracts associated with the preoral and postoral ciliated bands.

As the preoral, postoral and adoral ciliated bands developed, the oral hood was also beginning to form (Fig. 3A, B). Serotonin-like immunoreactivity was observed along the ciliated bands in the form of monopolar cell bodies with axonal-like tracts following the path of these bands (Fig. 3A). By this stage, a ganglion was evident at the anterior end of the larva. This anterior ganglion (AG) consisted of the immunopositive cells and processes innervating the preoral and postoral ciliated bands and processes interconnecting these bands (Fig. 3A).

Advanced bipinnaria (about 18 days old) underwent a

distinct shape change with the formation of an extension at the anterior end of the larva (Fig. 4A, B). The three ciliated bands were well developed in these larvae (Fig. 4A, B). Internally, the larva had a well-developed gut, and the right and left enterocoels had formed. The distribution of immunoreactive cells in these bands, discussed below, was consistent in all larvae examined ($n = 100$).

Adoral ciliated band

The adoral ciliated band was located along the posterior margin of the mouth and was characteristically paraboloidal (Fig. 5A). Along this band were densely packed cells with

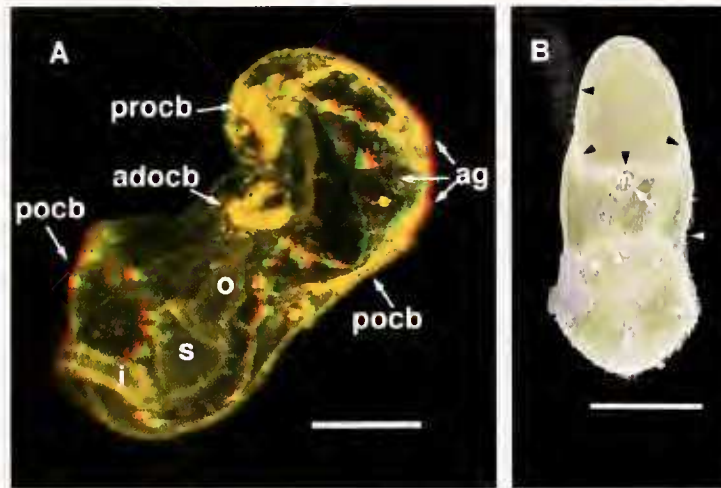


Figure 3. Three-dimensional red/green anaglyph and a scanning electron micrograph (false colored) of early bipinnariae. (A) A 3-D lateral view of an early bipinnaria showing immunoreactive cell bodies and axonal-like tracts following the ciliated bands. The anterior ganglion (ag) has formed and connects the preoral (procb) and postoral (pocb) ciliated bands. adocb, adoral ciliated band; o, esophagus; s, stomach; i, intestine. Bar, 95 μm . (B) Ventral view of an early bipinnaria at the same stage as in panel A. The ciliated bands are developing, but the anterior extension has not yet formed. Black arrowheads, preoral ciliated band; white arrowheads, postoral ciliated band; white arrow, adoral ciliated band; m, mouth; a, anus. Bar, 100 μm .

5-HT-like immunoreactivity; the apical ends of these cells extended to the edge of the ciliated epithelium. These cells were pyramidal and connected basally *via* a thick immunopositive tract (Fig. 6A). Compared with the other ciliated bands, the adoral ciliated band had the highest concentration

of immunoreactive cells and processes, forming the adoral nerve plexus (ADNP). The ADNP innervated the epithelium of the adoral ciliated band. Confocal optical sectioning revealed that the apical region of these cells protruded to the exterior of the ciliated band epithelium. Computer animations (data not illustrated) and a 3D anaglyph (Fig. 5A) showed that this plexus was also connected by serotonergic processes with the nerve plexus in the preoral transverse band *via* two thin (approximately 2.5 μm) lateral immunoreactive tracts.

Preoral ciliated band

The preoral ciliated band was located on the ventral surface of the larva and outlined the oral hood (Fig. 4A). Where this band traversed the larva above the mouth (preoral transverse region), a large number of flask-shaped cell bodies with 5-HT-like immunoreactivity ($\bar{x} = 21$, SE = 0.1 $n = 10$ larvae) were found in the epithelium (Fig. 4B). From these cell bodies, confocal sectioning into the larva from the ventral surface revealed axonal-like processes from the basal portion of the cells extending inwards toward the buccal cavity (Fig. 4B). In its lateral region, the preoral ciliated band contained a few immunopositive cells scattered along its path. Occasionally, a collection of cell bodies forming a pair of lateral ganglia were seen in the lateral region of the postoral ciliated band. These structures were not seen in all larvae and appear to be ephemeral. In the late bipinnaria, a ganglion developed at the anterior end of the larva. This ganglion consisted of immunoreactive cells

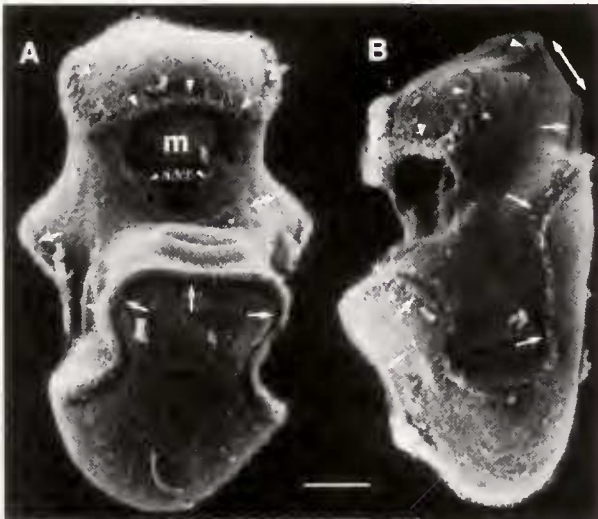


Figure 4. Scanning electron micrographs of a bipinnaria showing fully developed ciliated bands (arrowheads and arrows); (A) Ventral view of a bipinnaria with a flexed oral hood and mouth (m) open showing the position of the adoral ciliated band (adcb). (B) Lateral view of a bipinnaria showing the anterior extension of the oral hood, top right-hand side. Double-ended arrow indicates the anterior region where the anterior ganglion links the preoral and postoral ciliated bands. Arrowheads, preoral ciliated band; arrows, postoral ciliated band; Bar, 200 μm .

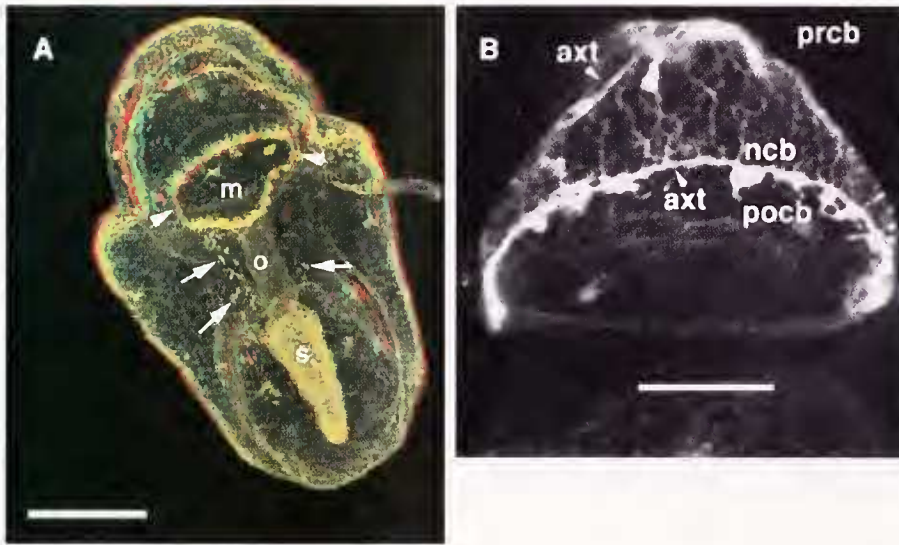


Figure 5. Bipinnaria: 3-D anaglyph and a high-magnification confocal image projection of the anterior ganglion. Images were constructed from a series of optical sections covering a distance of 132 μm . (A) 3-D anaglyph of a bipinnaria detailing the serotonergic nervous system following the pathway of the ciliated bands. White arrowheads, serotonergic connection between preoral and adoral ciliated bands; s, stomach; m, mouth; o, esophagus; arrows, immunoreactive coelomic cells. Bar, 200 μm . (B) A projection of the anterior ganglion in a late bipinnaria. Parallel axonal-like tracts on the opposing sides of the preoral ciliated band (prcb) and the postoral ciliated bands (pocb) interconnecting in a fine network of processes with 5-HT-like immunoreactivity. axt, axonal-like processes; ncb, nerve cell bodies. Bar, 50 μm .

associated with the preoral and postoral ciliated bands and a network of varicose processes spanning the two bands. This structure innervated the two bands and is a prominent neuroanatomical feature in the bipinnaria of *Patriella regularis* (Fig. 5B).

Postoral ciliated band

The postoral ciliated band formed a continuous loop traversing the ventral surface adjacent to the mouth and continued laterally along either side of the larva (Figs. 4A, B; 5C). It then extended posteriorly, crossing to the dorsal surface and then extending anteriorly, where it formed part of the AG (Fig. 4A, B). Cells and axonal-like processes with 5-HT-like immunoreactivity occurred along the band (Fig. 5A, C). In the transverse region adjacent to the mouth, a group of immunoreactive ovoid cells were connected by a thin immunopositive tract (Fig. 6C). In general, the immunoreactive cells and processes were more sparsely distributed in the postoral ciliated band than in the other bands (Fig. 6A–C).

Numerous cells of the left and right coelomic pouches exhibited 5-HT-like immunoreactivity (Fig. 5A). These cells were not neuronal in morphology, but they appeared to be exhibiting a general expression for serotonin. Immunoreactive cells were also concentrated around the anal opening (Fig. 7) and were observed in the intestinal epithelium, extending as much as 100 μm towards the stomach. In some

instances the entire stomach was immunoreactive, but this was not consistent and may have been due to incorporation of algal pigments (Fig. 7).

Discussion

Serotonergic cells were first seen at the animal pole of the gastrula of *Patriella regularis*. Similar findings have been reported for other asteroids and echinoids with planktotrophic development (Bisgrove and Burke, 1986; Nakajima, 1988). As the gastrula elongated, these cells formed a hemispherical array at the anterior end of the larva, indicating the onset of migration and rearrangement of these cells. Cell-fate studies would be required to determine cell migration and rearrangement. The neuronal-like cells present in the gastrula included monopolar, bipolar, and tripolar cells. The cell bodies of the monopolar and bipolar neurons appeared to be flask shaped, similar to the cell bodies described for neurons in the bipinnaria larvae of an asteroid (Lacalli *et al.*, 1990). Flask-shaped nerve cells have also been observed in an echinoid pluteus (Bisgrove and Burke, 1986; Bisgrove and Raff, 1989). Burke (1983) noted that nerve cells in the bipinnaria of the asteroid *Pisaster ochraceus* had a fusiform profile. We did not observe any neuron-like cells in *P. regularis* with a similar shape. Three-dimensional reconstructions of the tripolar cells revealed that they were pyramidoid, a structure not previously reported. This observation would, however, be dependent on the imaging

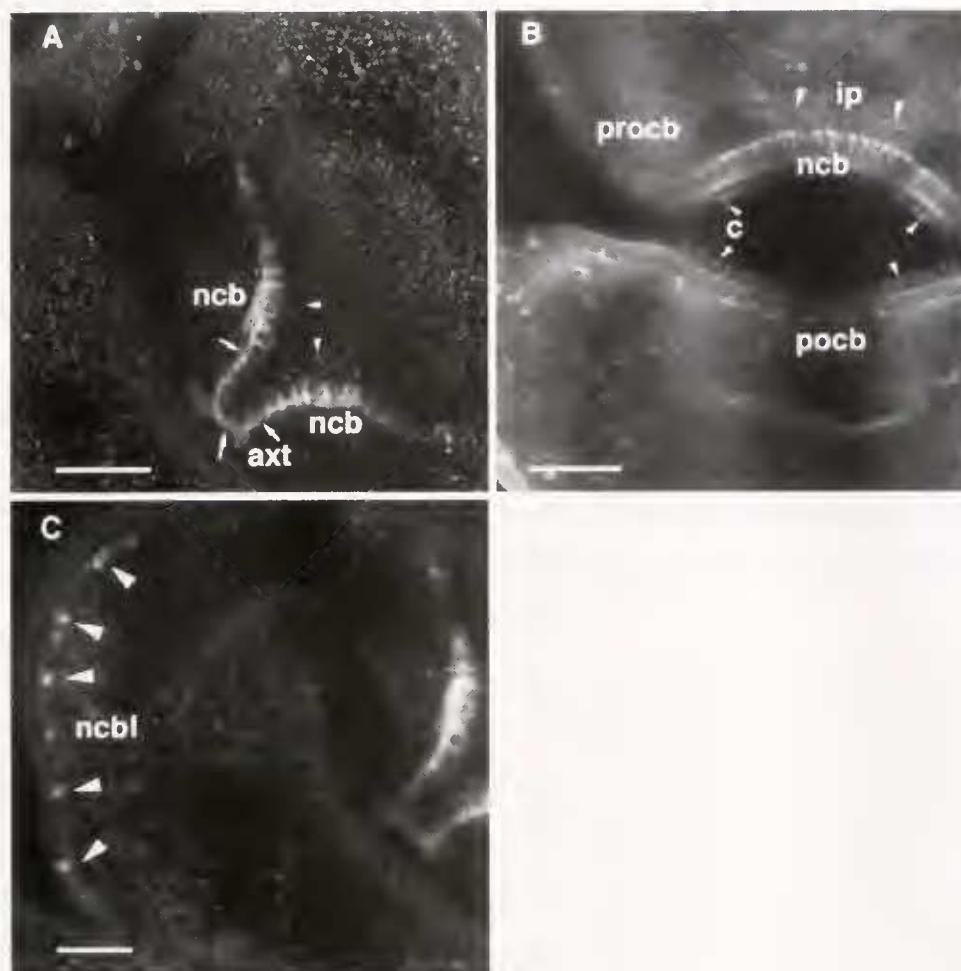


Figure 6. Confocal images detailing immunoreactive cells in ciliated bands. (A) Image from 15 optical sections (total thickness 139 μm) showing nerve cell bodies (ncb) with 5-HT-like immunoreactivity and an axonal-like tract (axt and arrows) in the adoral ciliated band. The entire band is immunoreactive. Note that the apical region of the neuron-like cells extend to the edge of the epithelium of the ciliated band. Arrowheads, cilia projecting into the buccal cavity. Bar, 20 μm . (B) The preoral and postoral ciliated bands of a fully developed bipinnaria. Note the greater number of cells with 5-HT-like immunoreactivity (ncb) present in the preoral ciliated band (procb) compared with the postoral ciliated band (pocb). The preoral ciliated band has immunoreactive processes (ip and lateral arrowheads) extending towards the buccal cavity. c and arrowheads, cilia. Bar, 63 μm . (C) Nerve cell bodies (ncbl and arrowheads) in the right lateral postoral ciliated band. Bar, 50 μm .

technique employed. Monopolar cells were the most common type of immunoreactive cell in the preoral and postoral ciliated bands, whereas multipolar pyramidal cells were the most common cell type in the adoral nerve plexus (ADNP) of the adoral ciliated band.

The presence of the apical projection arising from the cell bodies in the ADNP suggests that this plexus may have a sensory role. This interpretation is similar to that of Komatsu *et al.* (1991), who defined sensory neurons in the bipinnaria of *Luidia senegalensis* as neurons whose apical surface contacts the external environment. Strathmann (1975) demonstrated that the cilia of the adoral ciliated band in bipinnariae are involved in carrying food particles into the esophagus. It is possible that the adoral ciliated band of

P. regularis plays a gustatorial function under the influence of serotonergic activity in the ADNP.

In the bipinnaria of *P. regularis*, serotonin-like immunoreactivity was conspicuous in the adoral ciliated band, in the preoral and postoral ciliated bands, and in the anterior ganglion. The adoral ciliated band was strongly fluorescent and connected to the preoral ciliated band by an immunoreactive tract. Detection of this connection was possible through generation of 3D anaglyphs from confocal optical sections, which allowed visualization, and tracing of the complex immunostained network with respect to larval anatomy. On-screen animations (Chee and Byrne, 1997) were also employed to view immunolabeled larvae to determine the structure and direction of the immunolabeled

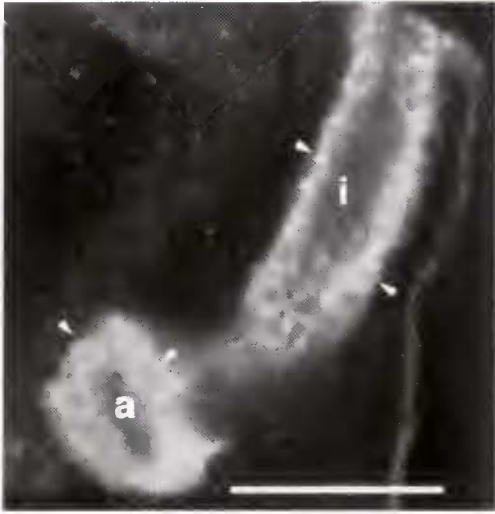


Figure 7. Confocal image showing immunoreactive cells (arrowheads) in the intestinal wall (i) and surrounding the anus (a); Bar, 50 μm .

processes. Although serotonergic immunoreactivity of the adoral ciliated band has been described in several studies (Nakajima, 1988; Moss *et al.*, 1994), the connections between the adoral and preoral ciliated bands have not been seen before. The conventional epifluorescence microscopy used in these earlier studies would not, however, have allowed resolution of this fine structure. Our observations demonstrate the presence of an extensive serotonergic communication network that connects all the ciliated bands and may govern reactions to stimuli and generate the behavioral patterns associated with feeding and swimming.

Optical sections through the oral region revealed that the immunoreactive cells in the preoral ciliated band gave rise to basal immunoreactive processes that project dorsally along the roof of the buccal cavity. The high density of immunoreactive cells in the region of the preoral ciliated band along the buccal opening suggests that these cells may play a sensory role in feeding. Selection and rejection of particles during feeding is thought to be associated with sensory cells in the buccal cavity (Strathmann, 1975). The cell processes in the roof of the buccal cavity in the larvae of *P. regularis* may connect with receptor sites that lie within the buccal cavity and are involved in particle selection in feeding. The 5-HT immunopositive tract connecting the adoral and preoral ciliated bands indicates a serotonergic link between the adoral ciliated band and the preoral ciliated band; this link could be important in feeding.

The anterior ganglion (AG) is first seen in early bipinnaria prior to formation of the anterior extension. As this extension develops, the ganglion becomes more intricate, forming a highly complex network. In advanced bipinnaria the AG consisted of prominent, strongly fluorescent tracts traversing the anterior region of the preoral and postoral

ciliated bands. The anterior ganglion was the only serotonergic connection between these ciliated bands.

In contrast to that suggested for sea stars (Lacalli, 1994), the AG of *P. regularis* does not split to form a pair of lateral ganglia. On the contrary, the pair of lateral ganglia occasionally seen in *P. regularis* (Chee and Byrne, 1997) are distinct from the AG. This bilateral collection of nerve cells may be homologous to those seen in other sea star larvae (Nakajima, 1988; Moss *et al.*, 1994), and it also corresponds to the position of peptidergic GFNSALMFamide (S1) ganglia seen in *P. regularis* (Byrne *et al.*, 1999).

At the brachiolaria stage, the AG is incorporated into the attachment complex, which contains many serotonergic neuronal-like cells suggested to be involved in the settlement process (Chee and Byrne, 1999). What appears to be a serotonergic AG has been observed in other echinoderms. Immunocytochemical labeling with anti-serotonin in the auricularia larvae of a holothuroid produced a structure described as an apical ganglion (Burke *et al.*, 1986). Unlike the AG of *P. regularis*, this structure was not composed of many immunoreactive tracts. Serotonergic AG that differ structurally from that of sea stars but are still anterior in position have been extensively described for sea urchin plutei (Bisgrove and Burke, 1987; Bisgrove and Raff, 1989; Nakajima *et al.*, 1993).

The AG in the bipinnaria of *P. regularis* is similar to the anterior concentration of serotonergic neurons, variously called apical organs or apical ganglia, characteristic of many invertebrate larvae (Lacalli, 1994). These appear to be highly conserved structures in marine invertebrate larvae and are thought to have a sensory function (Lacalli, 1994; Marois and Carew, 1997). The function of the AG and the significance of the connection between the preoral and postoral ciliated bands of *P. regularis* are not known. The position of the AG, considered together with the bipinnaria's anterior direction of swimming, suggests that it may have a sensory role in directional swimming; a similar function has been suggested for the apical ganglion of other invertebrate larvae (Marois and Carew, 1997). Moreover, in an ultrastructural study of the bipinnaria of *Luidia senegalensis*, sensory cells were found in the preoral and postoral ciliated bands (Komatsu *et al.*, 1991) in the region where the AG is located in *P. regularis*. Immuno-electron microscopic examination of thin sections from the anterior region of *P. regularis* would be needed to determine whether similar cells are present in this species.

The anterior ganglion in *P. regularis* is also similar to non-serotonergic neuronal structures in other asteroids. Similar catecholaminergic anterior structures in the bipinnaria of *Archaster typicus* were described as a "fluorescent anastomosis" (Chen *et al.*, 1995). Nakajima (1987) described a similar catecholaminergic structure as a "fibrous network" in the bipinnariae of *Asterias amurensis*. We

believe that confocal imaging would reveal that these structures are similar to the AG in *P. regularis*.

This study presents the most detailed immunocytochemical description of the development of the serotonergic system in a larval sea star. The organization of the serotonergic nervous system in the bipinnaria of *Patriella regularis* reflects the bilateral symmetry of the larva. A striking bilateral symmetry is also seen in the S1-like peptidergic system in *P. regularis* (Byrne *et al.*, 1999). For a complete picture of the expression of serotonin in nerve-like cells during development, we will continue this study in the brachiolaria of *P. regularis* through metamorphosis. Interestingly, serotonin has never been localized immunocytochemically in the nervous system of adult sea stars. It appears that complex serotonergic innervation is a feature common to the swimming and feeding larval form across a range of marine invertebrate phyla. Changes in expression of serotonin in the lecithotrophic larvae of the other *Patriella* species are being examined to document the evolution of neurogenesis in these asteroids.

Acknowledgments

We thank Paulina Selvakumaraswamy, Anna Cerra and Paula Cisternas, and Gillian Anderson for their comments and help with the manuscript. Ray Ritchie kindly supplied the algal cultures. Tony Romeo at the Electron Microscope Unit at the University of Sydney also provided assistance. This work was supported by an Australian Research Council grant.

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